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Differentiating the geographical origin of Ethiopian coffee using XRF- and ICP-based multi-element and stable isotope profiling

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ABSTRACT

To test the potential of different analytical tools to determine the geographical origin of Ethiopian coffee, 103 green arabica coffee samples from four coffee regions in Ethiopia were subjected to multi-elements and $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ determinations. Multi-elements were determined by using inductively coupled plasma (ICP)- and X-ray fluorescence spectrometry (XRF)-based techniques, and $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ were determined by using elemental analyzer-isotope ratio mass spectrometry. Using linear discriminant analysis, XRF-based multi-elements with and without $\delta^{13}C$ appeared to be most effective in discriminating the geographical origin of coffee, giving higher classification accuracy (89 and 86%, respectively) than ICP-based multi-elements with and without stable isotopes (80%, each). These results demonstrate the potential of XRF-based multi-element profiling as a relatively fast and low-cost tool to trace the geographical origin of Ethiopian coffee. All together this study offers the proof of concept for a promising method that, upon standardization, could be used for coffee provenance authentication and fraud detection.

1. Introduction

Food commodities with high quality and geographical indication of their origin are important for the world market and buying decisions of consumers (Kelly, Heaton, & Hoogewerff, 2005). This is also important for coffee, particularly for so-called 'specialty' coffee and 'single-origin' coffee. The consumer demand for authentic single-origin coffees of high quality is continually increasing, which might also increase the risk of fraud (Teuber, 2007; Valentin & Watling, 2013). In order to receive higher prices, there are often attempts to deliberately mislabel and pass off cheaper coffees or a mix of coffees as pure expensive specialty coffees (Anderson & Smith, 2002). This illustrates the importance of accurately identifying the origin of coffee. This is particularly important for Ethiopian coffee as: (1) coffee plays an important role in the country's economy, e.g., it constitutes about 25–30% of the country's total foreign currency earnings (Tefera, Tefera, & Gray, 2014) and provides a livelihood for at least 15 million people in the country (Gole, 2015); (2) the country produces distinct coffee types (e.g., *Harar*, *Yirgacheffe*, *Sidamo*, *Limmu* and *Lekemt coffee*), which are well recognized by the world market, in three major coffee regions varying in agroecology and/or coffee production system (Sereke-Brhan, 2010); and (3) there is a price difference and a trade-marking and licensing initiative for Ethiopian fine coffees based on their growing regions (Teuber, 2007).

So far, several research attempts have been carried out to find reliable methods that can correctly differentiate coffee provenance. Over the last two decades, determinations of biochemicals (Choi, Choi, Park, Lim, & Kwon, 2010; Mehari et al., 2016a, 2016b), multi-elements (Krivan, Barth, & Morales, 1993; Mehari, Redi-Abshiro, Chandravanshi, Combrinck, & McCrindle, 2016c), stable isotope ratios (Rodrigues et al., 2009; Serra et al., 2005) and a combination of these approaches (Bertrand et al., 2008; Liu, You, Chen, Liu, & Chung, 2014; McLeod, Garland, Hale, Steiman, & Frew, 2013), with several statistical techniques, have been proposed to differentiate the geographic origin of

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coffee at different spatial scales. This is because chemical constituents and their contents in coffee and other agro-products often differ based on growing environment (Gonzalvez, Armenta, & de la Guardia, 2009; Kelly et al., 2005). Promising results with varying degrees of classification and prediction success have been obtained. Recent studies (Mehari et al., 2016a, 2016c) obtained better classification and prediction success for Ethiopian coffee by using an approach relying on multi-element determination, rather than on biochemical properties (e.g., alkaloids) that may also be more prone to changes during post-harvest processing, storage and transport (Anderson & Smith, 2002). Bertrand et al. (2008) reported similar results for Colombian coffee. Consequently, these authors concluded that multiple element profiling is the method of choice for coffee origin determination. This is also supported by the findings of several other studies that tested the multi-element approach for coffee provenance differentiation at different spatial scales (Anderson & Smith, 2002; Habte et al., 2016; Muniz-Valencia, Jurado, Ceballos-Magana, Alcazar, & Diaz, 2014; Valentin & Watling, 2013). For multi-element determination, the above-mentioned studies mainly used different atomic absorption spectrometry (AAS) methods, inductively coupled plasma-optical emission spectroscopy (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS). These techniques require the solid samples to be digested with acids and follow time-consuming digestion procedures. Alternative techniques, such as X-ray fluorescence spectrometry (XRF), are based on direct analysis of the total elemental composition of solid samples, thus, eliminating the complex and sometimes time-consuming digestion procedure with possible analyte losses due to incomplete leaching, and minimizing contamination risks. Also the use of expensive high-purity acids can be avoided in this context (McComb, Rogers, Han, & Tchounwou, 2014). Despite its faster and more cost effective analysis compared to AAS and ICP-based techniques, the XRF technique has, to our knowledge, not been used for analysis of multiple elements in coffee in order to differentiate coffee provenance. To the best of our knowledge, it has also not been very often used for testing geographic origin of other agro-products.

Some other studies (e.g., Liu et al., 2014; McLeod et al., 2013) using multi-element and stable isotope profiles reported that variability in the elemental contents is the best discriminator for assessing the region of origin of coffee samples from some coffee-producing countries in America, Africa and Asia. High classification accuracy (86%) was also achieved by elemental contents combined with $\delta^{13}C$ and $\delta^{15}N$ or $\delta^{2}H$ values (McLeod et al., 2013). Furthermore, B and Sr isotope ratios can provide additional information (Liu et al., 2014). It has been shown that the combination of C, N, O, S and Sr isotope analysis with multi-element analysis allowed differentiation of coffees from various Hawaiian coffee-growing regions (Rodrigues et al., 2011). It has also been shown that isotopic analysis of C, N and O is an effective method in verifying the geographical origin for specialty coffees on a local geographical scale in Brazil (Barbosa et al., 2014). Some of these variables (e.g., $\delta^{13}C$ and $\delta^{18}O$) strongly depend on genotype, altitude, latitude, (micro)-climate and water availability (Flanagan & Farquhar, 2014; Rodrigues et al., 2016; Yeh & Wang, 2001). Moreover, some of these factors might change in the context of global climate change, which can affect Ethiopian coffee quality and yield (Moat et al., 2017). Despite the importance of coffee to the Ethiopian economy and high risk of fraud for single-origin high quality coffees, a combined multi-element and isotope ratio approach has not yet been tested for Ethiopian coffee.

Considering (1) the importance of identifying a rapid, easy to use and relatively cheap analytical method for authentication of coffee provenance, (2) the existence of different Ethiopian coffee types related to their growing regions and (3) the lack of detailed studies on Ethiopian coffee origin using a combined approach of multi-element and stable isotope profiles, we determined element content and stable isotope ($\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$) composition of 103 green arabica coffee bean samples collected from four coffee regions of Ethiopia. Specifically, the aim of our study was (1) to test the potential of either XRF- or ICP-based multi-element profiling with and without IRMS-derived $\delta^{13}C$, $\delta^{18}O$ and $\delta^{15}N$ profiling for discriminating the geographic origin of Ethiopian coffee and (2) to develop a proof of concept to trace Ethiopian coffee based on growing region via a combined multi-element and stable isotope approach.

2. Materials and methods

2.1. Description of sampling regions

The samples of green arabica coffee beans originated from 45 districts located in three major and one minor coffee regions, namely eastern (Harar), southeastern, southwestern and northwestern Ethiopia, respectively (Fig. 1; Table 1). These coffee regions have a different agroecology and/or coffee production system.

The Harar coffee growing region exists at altitudes between ca. 1200 and 2100 m asl (Table 1). It has a dryer climate than the wet southeastern or southwestern regions and experiences a bimodal rainfall, with short rains from March to May and long rains from June to September (Abebe, 2010; Klingele, 1998). The mean annual total rainfall is about 800–1200 mm, which is low for coffee cultivation, and the mean temperature is about 16–20 °C (Moat et al., 2017). Late Paleozoic to Eocene sedimentary rocks (Abbate, Bruni, & Sagri, 2015), and leptosols, luvisols, vertisols, cambisols and calcisols (Dewitte et al., 2013), respectively, are the major geological materials and soil types existing in the Harar coffee region. The green coffee beans of this region, produced predominantly under a garden system (coffee growing mostly around homesteads with other crops, e.g., fruit crops, root crops, *chat*, *enset* and spices, and a low level of shade tree canopy cover), are exclusively unwashed and are known as *Harar coffee* (Boot, 2011).

In the southeastern region, the main coffee growing areas are situated between 1500 and 2300 m asl (Abebe, 2005; Boot, 2011). This coffee region has a bimodal rainfall, with a short rainy season from mid February to April and a main rainy season from June to October (Yilma, 2001). The mean annual total rainfall ranges from 1000 to 1600 mm and the mean annual temperature ranges from 16 to 20 °C (Moat et al., 2017). In the southeastern region, Neoproterozoic basement rocks and Oligo-Miocene Trap basalts are the major geological materials (Abbate et al., 2015), and luvisols, fluvisols, cambisols and leptosols are the major soil types (Dewitte et al., 2013). Coffee in this region is predominantly produced under a garden system and processed as washed coffee. It is designated as *Sidamo* and *Yirgacheffe coffee* for marketing purposes (Boot, 2011).

The major coffee growing areas in southwestern Ethiopia exist at altitudes between ca. 850 and 2200 m asl (Table 1). The climate in this coffee region varies from sub-moist tropical to hot and humid rainforest climate with a unimodal rainfall. The mean annual temperature ranges from ca. 15 to 21 °C and the mean annual total rainfall ranges from ca. 1500 to 2100 mm, well distributed over a period of 8 to 9 months between March and November (Moat et al., 2017; Tegegne, 2017). The dominant geological materials in this coffee region are Oligo-Miocene Trap basalts and Neoproterozoic basement rocks (Abbate et al., 2015). The soils of the region are moderately acidic and nitosols is the dominant soil type in the coffee growing areas (Dewitte et al., 2013). Semi-forest coffee (managed forest coffee with a high shade tree canopy cover) is the dominant coffee production system in this region, prevailing over the other systems in Ethiopia: forest (unmanaged 'wild') coffee, garden coffee (coffee growing with other horticultural crops) and plantation (modern commercial) coffee (for details, see Gole, 2015). The region produces both washed and unwashed coffee, with unwashed coffee constituting a larger share of the total production. The coffee is designated as *Limmu, Jimma, Kaffa, Bebeka, Tepi* and *Lekemt coffee* for marketing purposes (Boot, 2011; Table 1).

The main coffee growing area in northwestern Ethiopia is concentrated in the highlands (ca. 1500–2100 m asl) of the Metekel, Agew Awi and West Gojam Zones. This coffee area is the smallest coffee

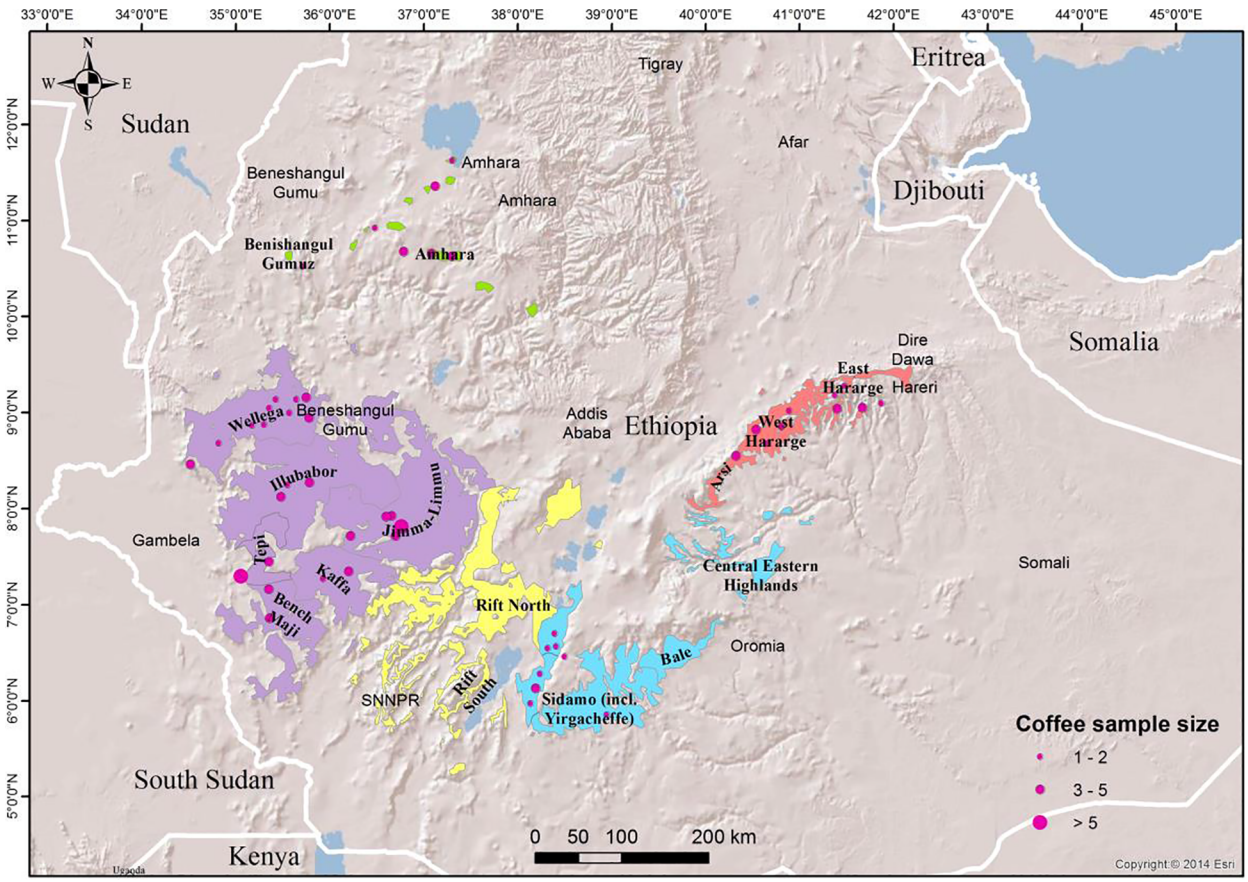

Fig. 1. Map showing the main coffee regions of Ethiopia (represented by colored polygons) and the sampling sites in four coffee regions (represented by deep pink colored dots within each colored polygon); red: Harar coffee region (West Hararge, East Hararge and Arsi); light blue: Southeast coffee region (Sidamo, Yirgacheffe, Bale and central eastern highlands); yellow: Rift Valley coffee region (Rift north and south); purple: Southwest coffee region (Jimma-Limmu, Kaffa, Bench Maji, Tepi, Godere, Illubabor and Wellega); green: Northwest coffee region (Amhara and Benishangul Gumuz). The size of the dots in each colored polygon indicates the sample size. This map is adapted from Moat et al. (2017). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

region of Ethiopia (Fig. 1), accounting for ca. 2% of the total land area covered by coffee, but it covers half of the minor coffee growing areas that are located in various parts of the country (Teketay, 1999). Mainly using a garden system, coffee production units in this region are also very small. The region has a unimodal rainfall with mean annual total values ranging from about 1400 to 1800 mm, distributed from May/June to August/September (Cheung, Senay, & Singh, 2008; Moat et al., 2017). In this coffee region, the mean temperature is about 16–19 °C (Fazzini, Bisci, & Billi, 2015), the geological materials are mainly Neoproterozoic basement rocks (Abbate et al., 2015) and the soil types are chiefly nitosols followed by cambisols (Dewitte et al., 2013). The coffee produced is exclusively unwashed and, until recently, it has been only used for local consumption.

2.2. Sample collection and preparation

Samples were collected in the 2010–2011, 2014–2015 and 2015–2016 cropping seasons from > 60 sites (45 districts), covering the four coffee regions of Ethiopia (Fig. 1). A total of 103 samples of green arabica coffee beans (each 1 kg), all prepared by dry processing (unwashed), were obtained from individual growers, large private farms, cooperative unions, local traders and coffee quality control units of Agriculture and Natural Resources Offices of the districts (Table 1). To obtain clean beans, defects, such as foreign matter as well as broken, black, faded, fungi affected and insect damaged, shrunken and immature beans were removed from each sample by handpicking. Then, clean beans of each sample were packed into a one kg plastic bag and stored at room temperature until analysis. A subsample of about 50 g beans per sample was dried at 40 °C until constant mass, and then ground into a particle size finer than 250 μm by using an M20 universal mill (IKA, Germany). Detailed information on the analyzed samples is given in Table 1.

2.3. Laboratory analysis

2.3.1. Multi-element determination by ICP-OES/ICP-MS

A total of 81 samples (14, 14, 38 and 15 samples from Harar, southeastern, southwestern and northwestern regions, respectively and collected in three cropping seasons: 2010–2011, 2014–2015 and 2015–2016) were used for multi-element determination by ICP-OES or ICP-MS. Per sample, a subsample of approximately 0.1–0.3 g was weighed and transferred into a Teflon beaker for digestion. In the first step of the digestion procedure, 2 ml of 14 M HNO_3 and 6 ml of 12.6 M HCl (both purified by in-house sub-boiling distillation) were added, and the beakers were placed for 12 h on a hotplate at 110 °C. The samples were not totally digested and therefore, after evaporating to dryness at 100 °C, the residues were taken up in 6 ml of 14 M HNO_3 and 1 ml of 9.8 M H_2O_2 (analytical grade) and submitted to a microwave-assisted acid digestion in Milestone MLS 1200. The microwave heating program consisted of 2 min irradiation at 250 W, 2 min at 0 W, 6 min at 250 W, 5 min at 400 W and 5 min at 600 W (total digestion time = 20 min). After complete digestion, in order to be sure acid concentrations are the same in all samples and standard solutions, the samples were evaporated to dryness at 100 °C, and the resulting residues were re-dissolved

Table 1
Summary information of the analyzed coffee samples (Alt. = altitude; Lat. = latitude; Lon. = longitude; n = number of samples).

Region	Coffee type	Sampling district	Alt. (m asl)	Lat. (°N)	Lon. (°E)	n
Harar (n = 25)	Harar	Bedeno	1540–2000	9.0924	41.6322	5
		Girawa	1750–2000	9.1370	41.8332	1
		Deder	1450–1800	9.3219	41.4463	1
		Mesela	1200–1800	9.2269	41.3472	2
		Melkabelo	1500–2000	9.0833	41.3667	3
		Odabultum	1800–2000	8.8942	40.7834	1
		Gemechis	1500–1600	8.9040	40.7817	1
		Boke	1762–1855	8.7227	40.6371	1
		Darolebu	1873–2000	8.5942	40.3061	4
		Habro	1600–2000	8.8667	40.5167	4
		Chiro	1826–2100	9.0674	40.8657	2
Southeast (n = 15)	Sidamo	Yirgalem	1800–1900	6.7360	38.4055	1
		Aleta-Wendo	1900–2000	6.6032	38.4193	1
		Aleta-Chuko	1900–2000	6.5828	38.3297	1
	Guji	Ana-Sora	1700–1800	6.4943	38.5084	1
		Adola-Rede	1700–1800	5.8850	38.9493	2
	Yirgacheffe	Wenago	1800–1900	6.3130	38.2530	2
		Yirgacheffe	1800–1950	6.1620	38.2058	5
		Kochere	1720–1850	6.0039	38.1531	2
Southwest (n = 48)	Bebeka	Bebeka[a]	850–1200	6.8835	35.4281	1
	Tepi	Tepi[a]	1100–1300	7.1870	35.4155	3
		Gemadiro[a]	1700–1800	7.4724	35.4172	3
		Godere	1350–1800	7.3187	35.1269	4
	Jimma	Gore[a]	1770–2085	8.1513	35.5357	1
		Metu	1600–1800	8.2787	35.6012	2
		Yayu	1200–2000	8.3031	35.8319	4
	Limmu	Gera	2000–2036	7.7502	36.2654	3
		Gomma I[a]	1660–1820	7.9619	36.6950	3
		Limmu-Kossa	1600–2000	7.8500	36.8000	6
	Kaffa	Lem-Kaffa[a]	1700–1820	7.3793	36.2492	3
		Woshi[a]	1700–1900	7.3000	35.9833	1
	Lekemt	Gimbi	1700–2200	9.1927	35.7894	4
		Haru	1672–1950	8.9800	35.8253	3
		Homa	1500–1800	9.0324	35.6174	2
		Guliso	1600–1700	9.1708	35.4727	1
		Lalo-Kile	1430–1667	8.9088	35.3508	1
		Hawa-Gelan	1700–1900	8.7068	34.8765	1
		Anfilo	1300–1800	8.4825	34.5847	2
Northwest (n = 15)	Gojam	Finote-Selam[a]	1800–1917	10.6824	37.3128	1
		Bure	2050–2091	10.7074	37.0998	2
		Mecha	1900–2100	11.4140	37.1305	5
		Zegie[a]	1770–1975	11.6884	37.3116	1
		Ankasha	1600–1860	10.7270	36.8042	4
		Chagni[a]	1650–1700	10.9730	36.4987	1
		Wenbera	1900–2097	10.5673	35.7440	1
Total n						103

[a] Bebeka, Tepi and Gemadro, Gore, Gomma I, Lem-Kaffa, Woshi, Finote-Selam, Zegie and Chagni are sampling sites located in the South Bench, Yeki, Ale, Gomma, Gewata, Chena, Jabi Tehnan, Bahir Dar Zuria and Guangua Districts, respectively.

in 1 ml of 0.4 M HNO_3. Next to the samples, a blank procedure was prepared using the same digestion procedure but without sample intake.

The elemental analysis was carried out first using a Spectro Arcos ICP-OES instrument with radial observation and using 32 linear CCD detectors in a Paschen-Runge mount for the simultaneous observation of the entire spectral range between 130 and 770 nm with a resolution of 3 pm at wavelengths < 340 nm and of 6 pm at wavelengths > 340 nm. All samples were diluted 100-fold with 0.4 M HNO_3 before measuring. With this instrument, elements present at a higher concentration (K, Ca and Mg) were determined, whereas for elements present at a lower concentration (Fe, Mn, Cu, Al, Rb, Sr, Ba, Ni, Cr, Mo, Co, La, Ce, Zn, B and Na), a quadrupole-based ICP-MS (XSeries 2, Thermo Scientific, Germany) and a sector-field ICP-MS (Element XR, Thermo Scientific, Germany) instruments were used after diluting all samples 500- or 1000-fold with 0.4 M HNO_3. Quantification was accomplished via external calibration while an internal standard (Yttrium) was relied on for correction of potential matrix effects, signal drift and instrument instability. The calibration standards were multi-element standards that have been prepared from commercially available stock solutions at different levels of concentrations. Repeatability was also evaluated by analyzing some samples in triplicate, with relative standard deviation shown to be generally < 10% for the elements of interest. The contents obtained were finally expressed in $\mu g\ g^{-1}$ dry coffee. Of the 19 elements determined, 7 elements (i.e., Mo, Co, La, Ce, Zn, B and Na) were not considered for statistical analysis as more than one sample per coffee type (Table 1) showed LOD (limit of detection) values for these elements.

2.3.2. Multi-element determination by WD-XRF

A total of 47 samples (11, 7, 20 and 9 samples from Harar, southeastern, southwestern and northwestern regions, respectively and collected in two successive cropping seasons: 2014–2015 and 2015–2016) were used for XRF analysis. Freeze-dried sample materials were ground using a standard coffee grinder prior to being mixed with a polypropylene wax binding agent (Ceridust 6050 M, Clariant, Switzerland) at a ratio of 1:4 (binder:sample). The sample materials were then pressed into 40 mm diameter pellets at 150 kN using a manual press (TP20, Herzog, Germany). Repeatability was assessed by analyzing selected samples in triplicate, with relative standard deviation < 10%

for the elements of interest. Multi-element determination (K, Ca, Mg, Al, Si, P, S, Cl, Fe, Mn, Cu, Zn, Rb, Sr and Ni) was carried out using a wavelength-dispersive X-ray fluorescence spectrometer (WD-XRF) (Axios Max, PANalytical, The Netherlands). In this multi-element determination, the values we obtained for some elements such as K, Ca, Mg, Al, Si, P, S and Fe were their oxide forms (i.e., K_2O, CaO, MgO, Al_2O_3, SiO_2, P_2O_5, SO_3 and Fe_2O_3). The instrument operated at 4 kW using an Rh target X-ray tube. During sequential analysis of elements, tube settings ranged from 25 kV, 160 mA for low atomic weight elements up to 60 kV, 66 mA for high atomic weight elements. All analyses were carried out using the Omnian analysis application (PANalytical, The Netherlands) and instrument drift was assessed following internal quality control procedures using a multi-element glass sample. This application is based upon theoretical matrix correction factors, i.e., it is not reliant upon matrix matched standards for the calibration. Elements that were not detected in (had no data for) more than two samples per coffee type (Table 1) were excluded from statistical analysis.

2.3.3. Stable isotope ratio determination

A total of 81 samples (14, 14, 38 and 15 samples from Harar, southeastern, southwestern and northwestern regions, respectively and collected in three cropping seasons: 2010–2011, 2014–2015 and 2015–2016) were used for the determination of C, N and O stable isotope ratios. The C and N stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) were analyzed via an elemental analyzer (ANCA-SL, SerCon, UK) interfaced with an isotope ratio mass spectrometer (IRMS) (20–22, SerCon, UK) and expressed relative to international standards; VPDB (Vienna Pee Dee Belemnite, IAEA) and AIR (N_2 in air, IAEA), respectively. A laboratory standard (wheat flour, $\delta^{13}C = -27.01 \pm 0.08‰$, $\delta^{15}N = +2.69 \pm 0.22‰$, average ± uncertainty toward secondary reference material, calibrated by ISO-Analytical, UK) traceable to IAEA-CH6 (accepted value = −10.449 ± 0.033‰) and IAEA-N-1 (accepted value = +0.4 ± 0.2‰) was used for data normalization. Periodic assessment of the instrument performance did not result in any significant scale stretch within the $\delta^{13}C$ and $\delta^{15}N$ range of the measured samples, for which normalization was done using the laboratory wheat flour standard as single anchoring point. The laboratory standard was analyzed in duplicate every 8 samples for drift correction, and a caffeine laboratory quality control sample was analyzed in the middle of drift standards, for quality assurance (deviation < 2·σ).

Samples for O stable isotope ratio ($\delta^{18}O$) analysis were prepared by weighing subsamples into silver foil capsules, after which the samples were dried at 105 °C for 24 h before being transferred to a desiccator, to cool down to room temperature before the silver cup was folded and kept in the desiccator until analysis. The $\delta^{18}O$ was determined using a thermal conversion element analyzer (SerCon, UK) interfaced with an IRMS (20–20, SerCon, UK) and expressed relative to VSMOW (Vienna Standard Mean Ocean Water, IAEA). Two secondary reference materials (i.e., USGS-34: $\delta^{18}O = +27.9 \pm 0.6‰$ and USGS-35: $\delta^{18}O = +57.5 \pm 0.6‰$) were used for scale normalization and a third secondary reference (USGS-32: $\delta^{18}O = -25.7 \pm 0.4‰$) was used for quality control (deviation < 0.8‰ from accepted value). USGS-34 and USGS-35 were analyzed alternatively every 8 samples to correct for instrument drift.

Amount of sample and reference material were matched to produce the similar beam areas (± 10%), samples were measured in duplicate and rejected (reanalyzed) is deviation exceeded 2·σ ($\sigma^{13}C = 0.2‰$, $\sigma^{15}N = 0.3‰$, and $\sigma^{18}O = 0.5‰$).

2.4. Statistical analysis

Linear discriminant analysis (LDA) was carried out for four datasets: (1) multi-element data as obtained by ICP-based techniques (ICP-based multi-elements), (2) multi-element data as obtained by ICP-based techniques and stable isotope ratio ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$) data (ICP-based multi-elements and stable isotopes), (3) multi-element data as obtained by WD-XRF (XRF-based multi-elements), and (4) XRF multi-element data as obtained by WD-XRF and $\delta^{13}C$ data (XRF-based multi-elements and $\delta^{13}C$). In the absence of certified reference material for all elements in our sample matrix, we had to assume that ICP- and XRF-measurement accuracy per element is similar in all individual coffee samples analyzed. This is reasonably compatible since all coffee samples were measured under standardized and identical conditions (i.e., the same sample preparation, equipment and operator). This assumption does not violate the LDA conceptual framework.

First, missing values in each dataset, i.e., 10 values for the five elements (MgO, Mn, Rb, Sr and Ni) in each XRF-based multi-element dataset with and without $\delta^{13}C$, and 1 value for $\delta^{18}O$ in ICP-based multi-element and stable isotope ratio dataset were replaced by average values for the coffee type (Table 1). Second, one-way analysis of variance (ANOVA) was carried out using the SPSS Version 16.0 (IPM Corp, USA) to test for significant differences between the mean contents of elements and compositions of stable isotopes of the different regions. The data were tested for normality and homoscedasticity using the Anderson-Darling and Bartlett's test ($P = 0.05$), respectively. Differences among regions were considered significant when $p < 0.05$. Boxplots and heatmap of correlations, respectively, were constructed for each variable per coffee region and per multi-element dataset to visualize the data structure and the relationship between the contents of different elements. Third, LDA for the four coffee regions was performed for the four datasets (LDA; R packages MASS). The LDA followed the recommendation that the total number of observations must be at least five times greater than the number of independent variables (Carter, Yates, & Tinggi, 2015). To achieve this, for example, 10 variables (Al_2O_3, SiO_2, SO_3, CaO, Fe_2O_3, Cl, Zn, Rb, Sr and $\delta^{13}C$) subset was selected in the initial XRF-based multi-element and stable isotope ratio ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$) dataset based on ccr_1^2 index, which is related to ROY first root (anneal, R packages subselect) (Cerdeira, Silva, Cadima, & Minhoto, 2015). The data of these variables gave us the fourth dataset (i.e., XRF-based multi-elements and $\delta^{13}C$). Fourth, k-fold (k = 10) cross-validation was applied to test the robustness of LDA classification using hundred replicates for which the average misclassification error rate was calculated. Fifth, the approximate proportion of observations that were incorrectly classified was calculated for each region from a matrix plot obtained after cross-validation for each of the four datasets.

As the contribution of the northwestern region to both national and export markets is negligible compared to the other regions, LDA and cross-validation tests were also repeated for three regions excluding the northwestern region using the same procedure as before. The $\delta^{13}C$ data were included in the 'ICP multi-elements' tracer set since it strongly depends on genotypes as well as on altitude, climate and water availability (Rodrigues et al., 2016), and some of these factors might change in the context of global climate change, which can affect Ethiopian coffee quality and yield (Moat et al., 2017).

The results are presented in LDA scatter plots, each containing the first two functions of the discriminant analysis and the shaded ellipse containing 50% of the observations. The LDA was performed with the R statistical software Version 3.4.0 by using MASS (Venables & Ripley, 2002) and Subselect packages (Cerdeira, Silva, Cadima, & Minhoto, 2015).

3. Results

3.1. Analysis of variance and data structure

Normality and homoscedasticity tests of the data gave compatible results ($P < 0.05$). Based on ICP-based multi-elements (Fig. 2a), coffee regions showed a significant ($P < 0.05$) difference in Ba, Ca, Cr, Cu, Mn, Rb and Sr contents. Southwestern coffee had higher (1) Rb and Sr contents compared to the other three regions, (2) Ca and Mn contents compared to southeastern and northwestern regions, and (3) Ba content compared to Harar and northwestern regions. Harar coffee also had a

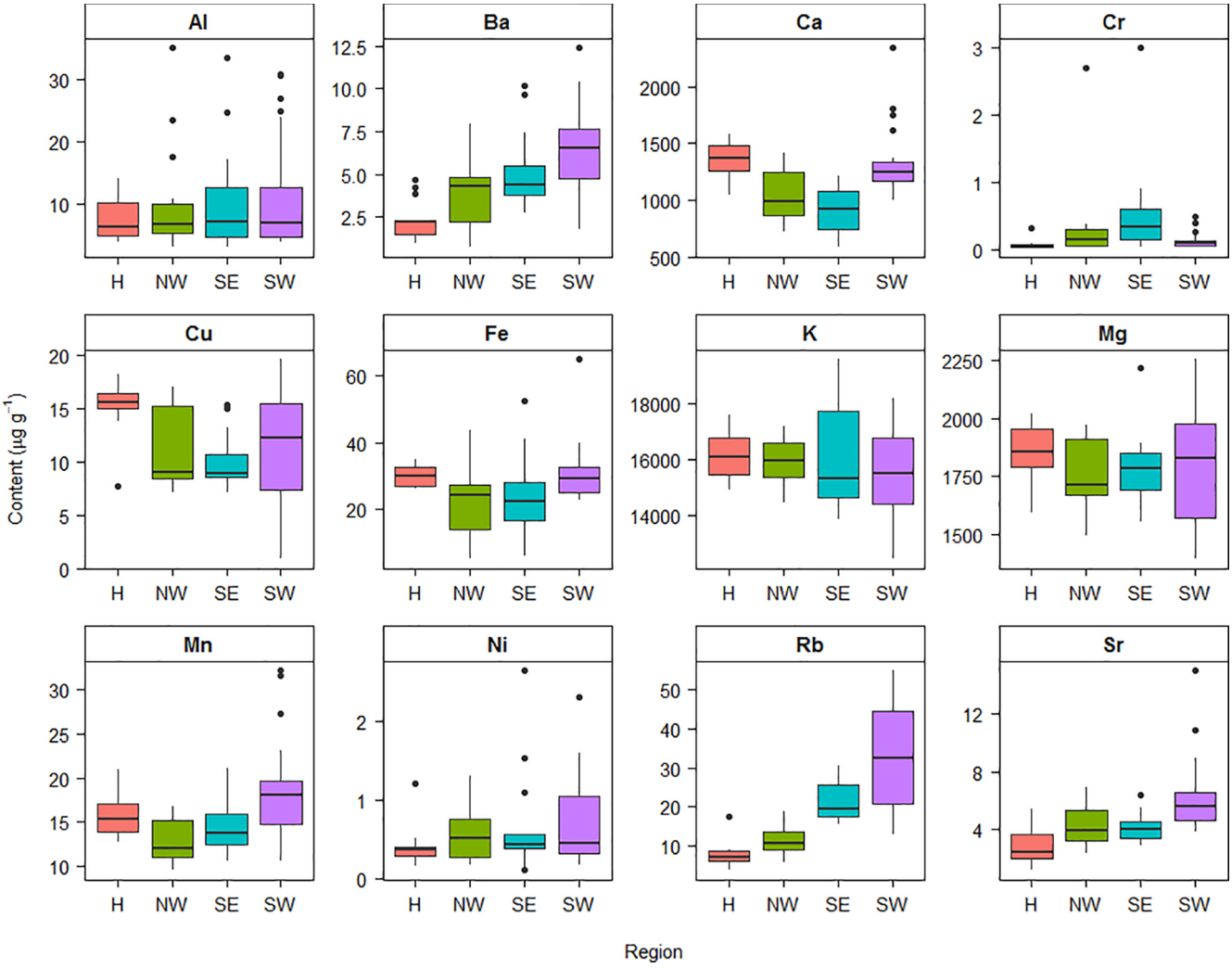

Fig. 2a. Boxplots of element contents of green arabica coffee beans from Harar (H), northwestern (NW), southeastern (SE) and southwestern (SW) coffee regions in Ethiopia and determined by ICP-OES or -MS (ICP-based multi-elements); lines within each box indicate the medians, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the interquartile range (IQR) from the 25th and 75th percentiles, individual dots beyond the whiskers indicate outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

higher Ca and Cu contents than the southeastern and northwestern regions, and all other regions, respectively, but it had a lower Cr content than all other regions. Rb content decreased consistently from the southwestern to southeastern to northwestern and to the Harar regions (Fig. 2a). As indicated by the heatmap correlations (Fig. S1a, Supplementary material), we also observed relatively high and positive correlations between Cu and Mg, Al and Fe, Sr and Ca, Ba and Sr, Ni and Mn, and Cr and Al, but high and negative correlations between Al and Mn, Ni and Cu, and Cr and Ca compared to between other elements.

Based on XRF-based dataset (Fig. 2b), coffee regions significantly ($P < 0.05$) differed for the contents of 9 elements out of 15 the elements determined. Harar and southwest coffees had higher contents for K_2O, MgO, P_2O_5 and SO_3 compared to northwest and southeast coffees, and southeast coffee for Al_2O_3 compared to Harar and northwest coffees. For CaO and Sr, Harar and southwest coffees, respectively, showed higher values, whereas southeast and Harar coffees, respectively, showed lower values than others. Harar coffee showed a higher Cl content than coffees of all other three regions. Again, Rb content decreased consistently from southwestern to southeastern to northwestern and to Harar regions (Fig. 2b). As indicated by the heatmap correlations in Fig. S1b, we also observed strong positive correlations between SiO_2 and Al_2O_3, P_2O_5 and MgO, SO_3 and MgO, SO_3 and P_2O_5, K_2O and MgO, K_2O and P_2O_5, and K_2O and SO_3, but relatively high and negative correlations between Rb and Cl, Rb and CaO, Sr and Cl, and Sr and CaO compared to between other elements.

The coffee regions also significantly ($P < 0.05$) differed for $\delta^{13}C$ and $\delta^{18}O$ values, but not for $\delta^{15}N$ values. The Harar coffee showed higher $\delta^{18}O$ values than the other regions, and Harar and southeastern coffees showed higher $\delta^{13}C$ values than southwestern coffee (Fig. 2c).

In summary, Harar and southwestern coffees contained higher contents of certain elements, with the exception of Al_2O_3, than those of the southeastern and northwestern regions. Harar coffee also showed higher $\delta^{18}O$ values than coffees of the other regions. Yet, no significant differences ($P > 0.05$) were observed between southeastern and northwestern coffees in their contents of the tested elements, except for Al_2O_3, CaO and Rb, and stable isotope ratios. Al and Sr from the ICP-based dataset, and MgO and K_2O from the XRF-based dataset showed strong positive correlations with two or more elements (Figs. S1a & b). This shows that, during coffee origin authentication, each of these elements from each respective multi-element dataset can substitute other elements that correlate with each of them.

3.2. Discriminant analysis between the four coffee regions

3.2.1. ICP-based multi-elements without and with stable isotope ratios

Figs. 3a & 3b, respectively, represents LDA plotted data of multi-

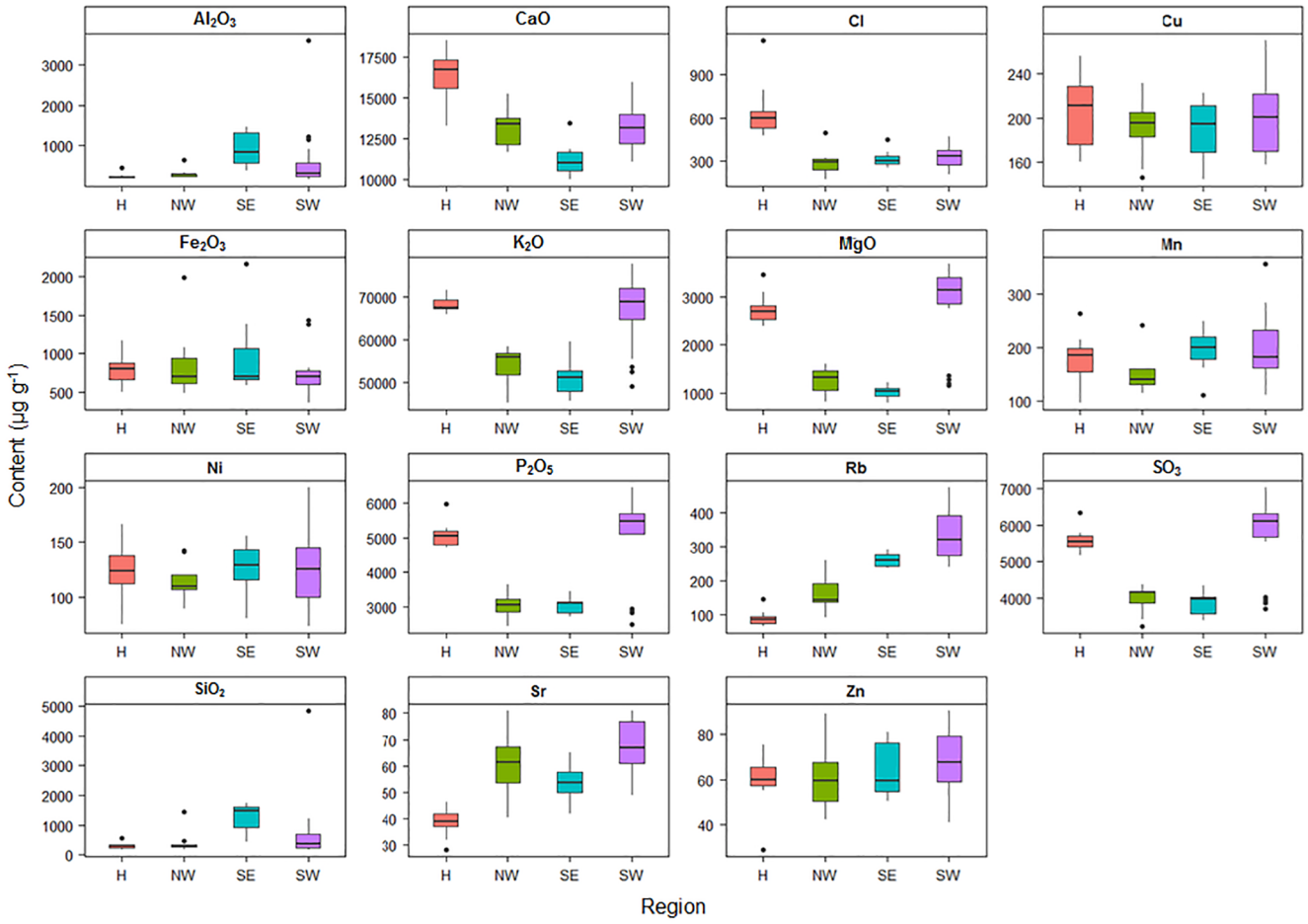

Fig. 2b. Boxplots of element contents of green arabica coffee beans from Harar (H), northwestern (NW), southeastern (SE) and southwestern (SW) coffee regions in Ethiopia determined by WD-XRF (XRF-based multi-elements); lines within each box indicate the medians, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the IQR from the 25th and 75th percentiles, individual dots beyond the whiskers indicate outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

element results as obtained by ICP-based techniques, and multi-element results as obtained by ICP-based techniques and stable isotope ratios. The first two functions of the discriminant analysis cover 98 and 97% of the variation of the data of the former and the latter datasets, respectively. Here, for both datasets, coffee samples did not show a clear separation based on their geographical regions of origin, particularly those of the northwestern and southeastern regions. Harar coffee, however, showed a good separation from coffees of the other three regions (northwestern, southeastern and southwestern) (Figs. 3a & 3b). The overall classification accuracy after cross-validation was ca. 80% for both datasets. For the ICP-based multi-element dataset, around 40 and 50% of the samples from the northwestern and southeastern regions, respectively, and for the ICP-based multi-element and stable isotope ratio dataset, around 33 and 36% of the samples from the northwestern and southeastern regions, respectively, were misclassified. This is much higher than those from the Harar and southwestern regions. For both datasets, for example, only one sample from Harar was misclassified.

3.2.2. XRF-based multi-elements without and with $\delta^{13}C$ values

Plotted LDA data of multi-element results as obtained by WD-XRF and multi-element results as obtained by WD-XRF and $\delta^{13}C$ values, respectively, is presented in Figs. 4a & 4b. The first two functions of the discriminant analysis cover 92 and 93% of the variation of the data of the former and the latter datasets, respectively. The plotted LDA data of both datasets showed that coffee samples were separated by their geographical regions of origin. Particularly, Harar, southeastern and southwestern coffee samples were markedly separated, whereas those of the northwestern and southwestern regions did not form very clear separate groups (Figs. 4a & 4b). The performance of LDA with XRF-multi-elements was satisfactory with an overall classification accuracy of 86%, and around 33% of the samples (3 of 9) from the northwestern region were misclassified. For the remaining regions, however, only one sample from each region was misclassified. The overall classification accuracy after cross-validation for the XRF-multi-elements and $\delta^{13}C$ values was about 89%. For this dataset, all Harar samples, around 78% of the northwestern samples (7 of 9) and around 71% of the southeastern samples (5 of 7) were correctly classified for the cross-validation model.

3.3. Discriminant analysis between the three major coffee regions

LDA of the three major coffee regions (Harar, southeastern and southwestern) using the multi-element data as obtained by WD-XRF with and without $\delta^{13}C$ values indicated a clear separation of coffee samples based on their geographical origins (Fig. 5a & b), with 88 and 80% overall classification accuracy after cross-validation for the former and the latter datasets, respectively. For both datasets, coffee samples from the Harar region formed a well-separated group with no misclassified samples for the cross-validation model compared to those from the other two regions (Fig. 5a & b).

Based on LDA of the multi-element data as obtained by ICP-based techniques with and without $\delta^{13}C$ values, coffee samples from these three regions were also separated based on their geographical origins

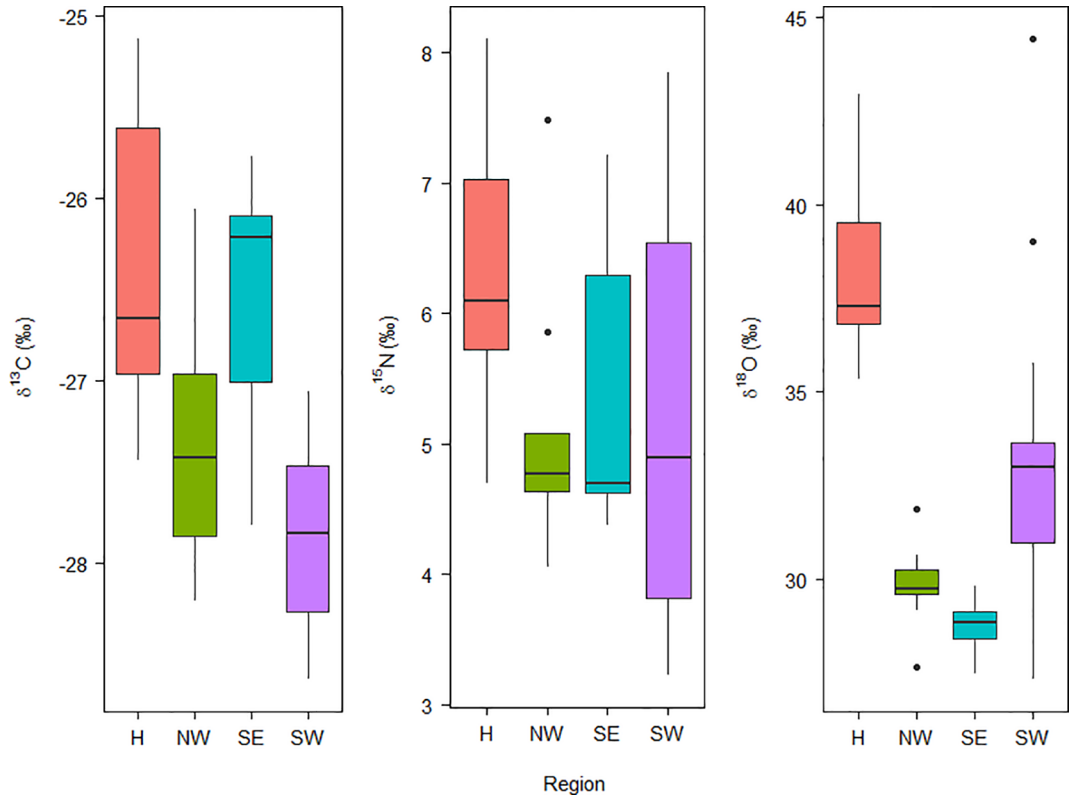

Fig. 2c. Boxplots of stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$) of green arabica coffee beans from Harar (H), northwestern (NW), southeastern (SE) and southwestern (SW) coffee regions in Ethiopia; lines within each box indicate the medians, box limits indicate the 25th and 75th percentiles, whiskers extend 1.5 times the IQR from the 25th and 75th percentiles, individual dots beyond the whiskers indicate outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

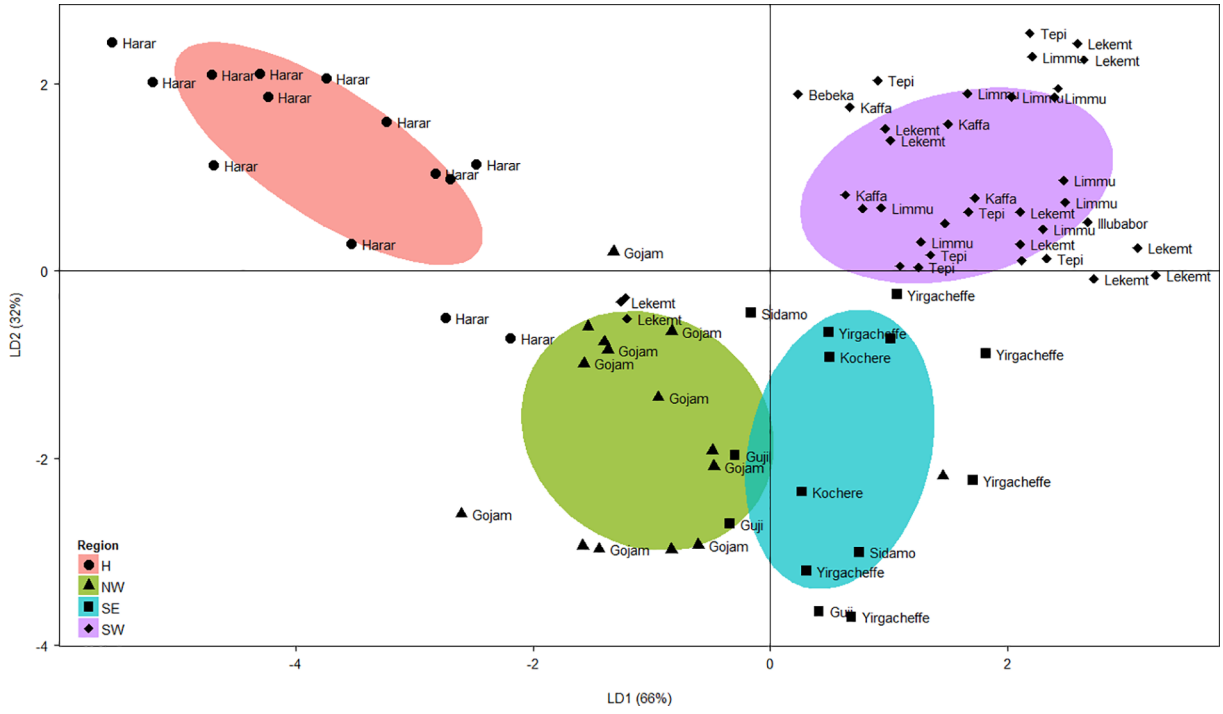

Fig. 3a. LDA plot of the first two discriminant functions (LD1 and LD2; 98%) showing the separation of coffee samples originating from Harar (H, red), northwestern (NW, green), southeastern (SE, blue) and southwestern (SW, purple) coffee regions of Ethiopia, based on the multi-element data as obtained by ICP-based techniques (ICP-based multi-elements); names behind symbols indicate the coffee types (see Table 1). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

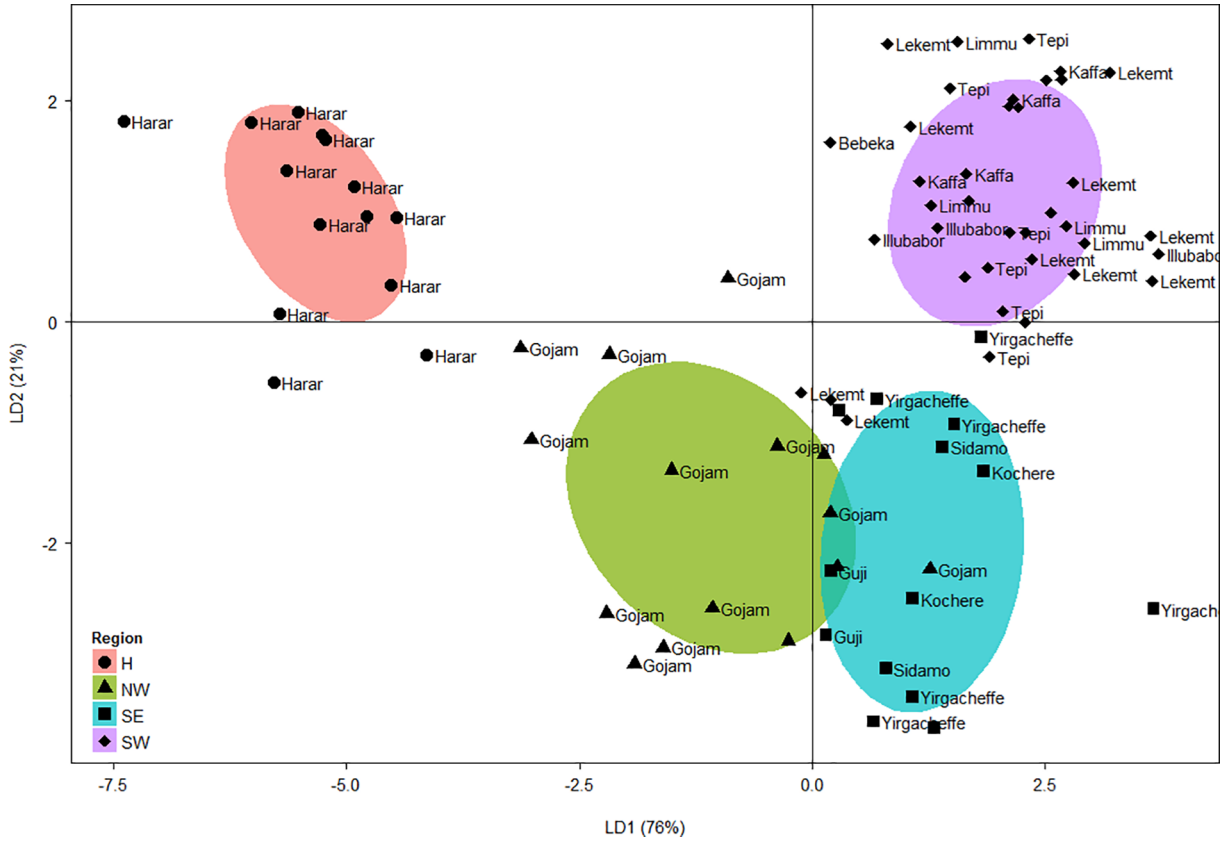

Fig. 3b. LDA plot of the first two discriminant functions (LD1 and LD2; 97%) showing the separation coffee samples originating from Harar (H, red), northwestern (NW, green), southeastern (SE, blue) and southwestern (SW, purple) coffee regions of Ethiopia, based on the multi-element data as obtained ICP-based techniques and the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values (ICP-based multi-elements and stable isotope ratios); names behind the symbols indicate the coffee types (see Table 1).

(Fig. 5c & d), with about 89 and 86% overall classification accuracy after cross-validation for the former and the latter datasets, respectively. All Harar coffee samples, except one sample for ICP data without $\delta^{13}C$, were correctly classified.

In general, higher numbers of samples were misclassified (i) when using ICP data than when using XRF data, (ii) without than with $\delta^{13}C$, and (iii) for southwestern coffee than for Harar and southeastern coffees.

4. Discussion

By using LDA of four datasets, namely (1) ICP-based multi-elements, (2) ICP-based multi-elements and $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$, (3) XRF-based multi-elements, and (4) XRF-based multi-elements and $\delta^{13}C$ of green arabica coffee bean samples from four coffee regions of Ethiopia, we demonstrated the possibility of discriminating Ethiopian coffee based on geographical origin with varying success for these different profiling methods. In general, the XRF-based multi-elements with or without $\delta^{13}C$, which is, to our knowledge, a new approach for coffee classification based on geographical origin, discriminated coffee samples according to their growing regions better than when using ICP-based multi-elements with or without $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$. Combining XRF-based multi-elements with $\delta^{13}C$ improved the classification accuracy of the coffee samples from the four coffee regions (Figs. 4a & 4b) only by 3% and from the three major coffee regions (Fig. 5a & b) only by 8%. Combining ICP-based multi-elements with stable isotope ratio information improved the classification accuracy of the coffee samples only for the three major coffee regions (Fig. 5c & d) by 3%. This may suggest that the coffee geographical origin differentiating power of XRF and ICP multi-element determination can slightly be improved by including stable isotope compositions. This improvement can be linked to variations between coffee samples from different regions in $\delta^{13}C$ and $\delta^{18}O$ (Fig. 2c). The $\delta^{13}C$ and $\delta^{18}O$ of agro-products including coffee depend on climate (e.g., rainfall, temperature, relative humidity and light intensity), distance between land surface and ocean, altitude, latitude, $\delta^{13}C$ values of source CO_2 and CO_2:O_2 ratio of the growing environment (Barbour, 2007; Flanagan & Farquhar, 2014; Yeh & Wang, 2001) while the multi-elements of agro-products reflect soil and climatic conditions of the growing environment (Gonzalvez et al., 2009; Kelly et al., 2005). This suggests that combining multi-element contents and stable isotope ratios of agro-products is expected to reflect growing regions than either of the two.

Literature on the discrimination of green arabica coffee beans, based on their element content and stable isotope composition, from a single country of origin is generally inadequate (Barbosa et al., 2014; Rodrigues et al., 2011). Some recent studies (e.g., Habte et al., 2016; Mehari et al., 2016a, 2016b, 2016c), however, attempted to classify Ethiopian coffee based on growing region by using ICP-based multi-elements or data on the contents of some selected biochemicals, e.g., chlorogenic acids and alkaloids, and an LDA statistical method. The overall classification accuracies we achieved for XRF-based or ICP-based multi-elements with $\delta^{13}C$ (88 and 89%) of the green coffee from three major coffee regions of Ethiopia (Fig. 5) were comparable to those achieved (92 and 93%) by Mehari et al. (2016c) and Muniz-Valencia et al. (2014) for ICP-OES multi-element data for green coffee from three major producing regions of Ethiopia and roasted coffee from four

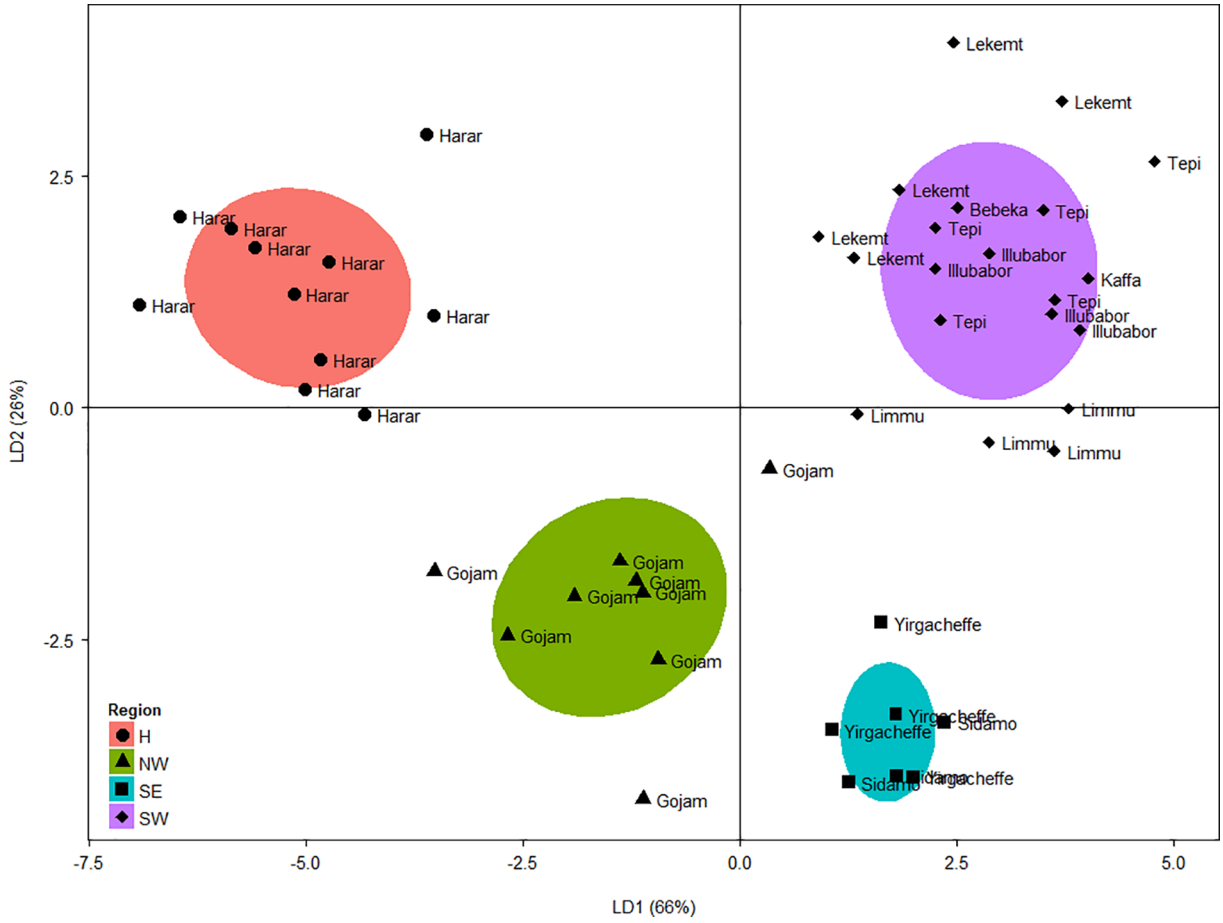

Fig. 4a. LDA plot of the first two discriminant functions (LD1 and LD2; 92%) showing the separation of coffee samples originating from Harar (H, red), northwestern (NW, green), southeastern (SE, blue) and southwestern (SW, purple) coffee regions of Ethiopia, based on the multi-element data as obtained by WD-XRF (XRF-based multi-elements); names behind the symbols indicate the coffee types (see Table 1).

producing states of Mexico, respectively. The overall classification accuracies we achieved for XRF-based multi-elements with $\delta^{13}C$ for coffee from three coffee regions (ca. 88%) were also similar with those achieved for ICP-based multi-elements with $\delta^{13}C$ (ca. 89%). However, the overall classification accuracies we achieved for XRF-based multi-elements with and without $\delta^{13}C$ (ca. 89 and 86%) of the coffee from four coffee regions were higher than those achieved for ICP-based multi-elements with and without isotope ratio (ca. 80%, each). This difference in classification accuracy can be linked to variations between type and contents of elements determined by using these techniques (Figs. 2a & 2b) and in correlations between different elements of the two techniques (Figs. S1a & b). This may in turn be due to their difference in sensitivity to detect and quantify different elements in agro-products. For example, the XRF technique, unlike the ICP technique, offers a means of rapid analysis of a wide range of total element concentrations in different matrices and is not subject to the constraints of chemical digests as in ICP-based methods. Considering the more rapid nature of XRF elemental analysis with lower risks of analyte loss and contamination (McComb et al., 2014) compared to those accompanying ICP or IRMS, these findings show the potential for using XRF technique for classification of (Ethiopian) coffee according to geographical origin.

The efficacy of chlorogenic acids and alkaloids as chemical descriptors for chemometric classification of the three major coffee growing regions of Ethiopia was also recently investigated (Mehari et al., 2016a, 2016b). The proportions of correct classification achieved using XRF-based or ICP-based multi-elements with $\delta^{13}C$ (88 and 89%) are also comparable to that achieved (90%) by Mehari et al. (2016b) using chlorogenic acids. But, the results of this and previous studies (Mehari et al., 2016c) indicate that application of multi-element analysis greatly improved classification accuracy compared to alkaloids, which provided only 75% correct classification (Mehari et al., 2016a). Moreover, chlorogenic acids and alkaloids determinations by HPLC or GC are more costly and follow more complex and time-consuming procedures than those for multi-element determination with XRF (Gonzalvez et al., 2009; Luykx & van Ruth, 2008). When comparing the three chemical approaches for classification of coffee based on geographic origin, chlorogenic acids and alkaloids may be more prone to changes during postharvest processing, storage and transport (Anderson & Smith, 2002), showing that the multi-elements approach is a more robust method for the classification of coffee growing regions from Ethiopia (Mehari et al., 2016c).

The clear separation of the Harar coffee compared to the other regions (Figs. 3–5) agreed with previous studies investigating the geographic origins of Ethiopian coffee at local (Habte et al., 2016; Mehari et al., 2016c) and country scales (Valentin & Watling, 2013) via multi-element data. This may indicate that Harar coffee is more traceable via multi-element determination with or without information on stable isotope ratios (e.g., $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$) compared to coffees from other regions in Ethiopia (Fig. 1). However, there is inconsistency between this and other studies regarding classification of coffee samples

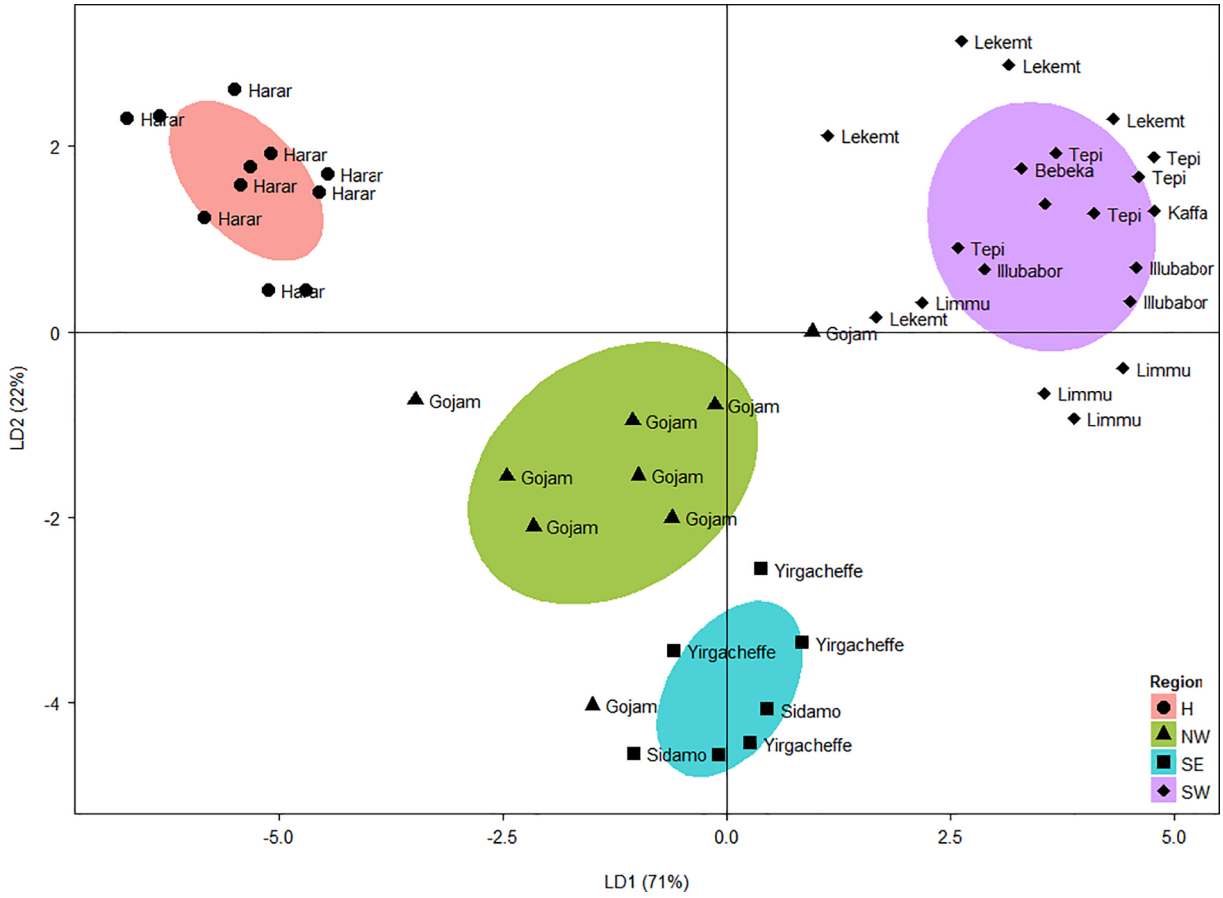

Fig. 4b. LDA plot of the first two discriminant functions (LD1 and LD2; 93%) showing the separation of coffee samples originating from Harar (H, red), northwestern (NW, green), southeastern (SE, blue) and southwestern (SW, purple) coffee regions of Ethiopia, based on the multi-element data as obtained by WD-XRF and $\delta^{13}C$ value (XRF-based multi-elements and $\delta^{13}C$); names behind the symbols indicate the coffee types (see Table 1).

from other regions according to their geographical origins particularly when all four coffee regions (Figs. 3 & 4) are considered. For example, in the LDA of ICP-based multi-elements to classify coffee samples according to their geographical origins at sub-regional level by Habte et al. (2016), samples from Harar (east), Gojam (northwest), Bale (southeast) and Bebeka (southwest) formed clearly separate groups, whereas samples from Yirgacheffe and Sidamo in the southeastern region, Tepi, Jimma, Illubabor, Limmu and Lekemt in the southwestern region were clustered together with unclear separation. However, southeastern and southwestern samples in our study formed relatively clear separate groups for all four datasets (Figs. 3 & 4), whereas those of southeastern and northwestern did not, particularly for ICP-based datasets (Figs. 3a & 3b). The results of LDA (Figs. 3 & 4) in our study were consistent with the ANOVA results revealing significant differences ($P < 0.05$) between Harar, southwestern, and southeastern and northwestern coffees for their mineral content and stable isotope ratio. However, no significant differences ($P > 0.05$) were found between southeastern and northwestern coffees. This difference between different regions' coffee can be linked to variations between coffee regions in geological material, soil type and climate (Table 1Abbate et al., 2015; Dewitte et al., 2013; Moat et al., 2017) as well as intrinsic water use efficiency of coffee genotypes.

An inconsistency between our (Fig. 5c) and a previous study (Mehari et al., 2016c) was also observed for incorrectly classified samples from three major coffee regions. In our study, four southeastern samples were incorrectly classified as southwestern coffee, three southwestern samples as Harar coffee, one southwestern sample as southeastern coffee and one Harar sample as southeastern coffee, whereas in Mehari et al. (2016c), only one sample from Harar was incorrectly classified as southwestern coffee. This difference may be related to the difference between the two studies in sample size and years of sample collection. For ICP-based multi-element dataset, for example, we collected 81 samples in three cropping seasons, whereas Mehari et al. (2016c) collected 49 samples in one cropping season.

5. Conclusion

For the first time, we demonstrated the potential of XRF-based multi-element data to discriminate samples of green arabica coffee beans according to their growing regions and provided proof of concept for tracing Ethiopian coffee based on growing region via multi-element and stable isotope profiling. Multivariate analysis (LDA) using XRF-based multi-elements for coffee samples from four coffee regions and three major regions provided 86 and 80% overall classification accuracy, respectively. This result obtained for the four regions is better than that obtained by ICP-based multi-element data (80%). Furthermore, coffee origin diagnostic power of XRF-based multi-elements can be refined further by including an additional tracer, i.e., $\delta^{13}C$ values of coffee that improved classification accuracy by 3 and 8% for four and three regions, respectively. In general, considering a more rapid, an easier and a cheaper element content analysis via XRF with no constraints of sample material chemical digests (i.e., ICP), these results indicate that the XRF-based multi-element approach can be a preferred method of choice to authenticate or determine the geographic origin of Ethiopian coffee and be applied to help combating fraudulent activities in the coffee market. However, this approach needs to be further standardized and therefore, a certified reference material for coffee should be developed.

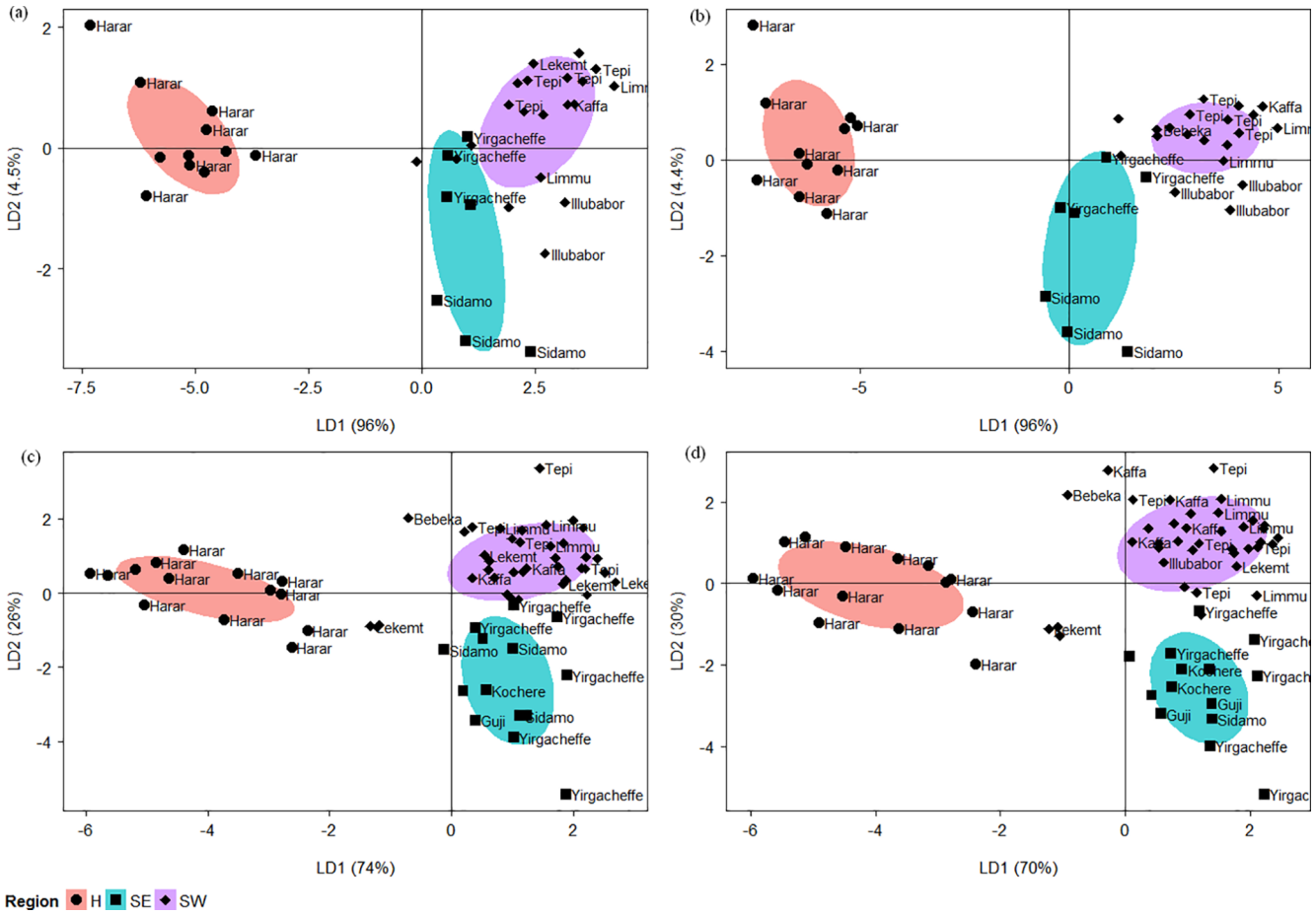

Fig. 5. LDA plot of the first two discriminant functions (LD1 and LD2; 100%) showing the separation of coffee samples originating from Harar (H, red), southeastern (SE, blue) and southwestern (SW, purple) coffee regions of Ethiopia, based on multi-element data as obtained by WD-XRF (XRF-based multi-elements) (a), multi-element data as obtained by WD-XRF and $\delta^{13}C$ values (XRF-based multi-elements and $\delta^{13}C$) (b), multi-element data as obtained by ICP-based techniques (ICP-based multi-elements) (c), and multi-element data as obtained by ICP-based techniques and $\delta^{13}C$ values (ICP-based multi-elements and $\delta^{13}C$) (d); names behind the symbols indicate the coffee types (see Table 1).

Acknowledgement

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Conflict of interest

The authors declare that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.03.135.

References

Abbate, E., Bruni, P., & Sagri, M. (2015). Geology of Ethiopia: A review and geomorphological perspectives. In P. Billi (Ed.). *Landscapes and landforms of Ethiopia* (pp. 33–64). Dordrecht: Springer Science + Business Media.

Abebe, T. (2005). *Diversity in homegarden agroforestry systems of southern Ethiopia.* PhD Thesis The Netherlands: Wageningen University and Research Center.

Abebe, D. (2010). Future climate of Ethiopia from PRECIS regional climate model experimental design. http://www.metoffice.gov.uk/media/pdf/o/9/PRECIS_Experimental_Design_Dawit.pdf (Accessed 09.03.2017).

Anderson, K. A., & Smith, B. W. (2002). Chemical profiling to differentiate geographic growing origins of coffee. *Journal of Agricultural and Food Chemistry, 50*, 2068–2075.

Barbosa, J., Borem, F., Alves, H., Cirillo, M., Sartori, M., & Ducatti, C. (2014). Discrimination of production environments of specialty coffees by means of stable isotopes and discriminant model. *Journal of Agricultural Science, 6*(5), 55–64.

Barbour, M. M. (2007). Stable oxygen isotope composition of plant tissue: A review. *Functional Plant Biology, 34*, 83–94.

Bertrand, B., Villarreal, D., Laffargue, A., Posada, H., Lashermes, P., & Dussert, S. (2008). Comparison of the effectiveness of fatty acids, chlorogenic acids, and elements for the chemometric discrimination of coffee (*Coffea arabica* L.) varieties and growing origins. *Journal of Agricultural and Food Chemistry, 56*, 2273–2280.

Boot, W. J. (2011). Ethiopian coffee buying manual: Practical guidelines for the purchasing and importing of Ethiopian specialty coffee beans. https://bootcoffee.com/wp-content/uploads/2013/01/Ethiopian_Coffee_Buying_Guide.pdf (Accessed 18.02.215).

Carter, J. F., Yates, H. S. A., & Tinggi, U. (2015). Isotopic and elemental composition of roasted coffee as a guide to authenticity and origin. *Journal of Agricultural and Food Chemistry, 63*(24), 5771–5779.

Cerdeira, J. O., Silva, P. D., Cadima, J., & Minhoto, M. (2015). Subselect: Selecting variables subsets. R package version 0.12-5. http://CRAN.R-project.org/package=subselect (Accessed 20.05.2016).

Cheung, W. H., Senay, & Singh, A. (2008). Trends and spatial distribution of annual and seasonal rainfall in Ethiopia. *International Journal of Climatology, 28*, 1723–1734.

Choi, M.-Y., Choi, W., Park, J. H., Lim, J., & Kwon, S. W. (2010). Determination of coffee origins by integrated metabolomic approach of combining multiple analytical data. *Food Chemistry, 121*, 1260–1268.

Dewitte, O., Jones, A., Spaargaren, O., Breuning-Madsen, H., Brossard, M., Dampha, A., ... Zougmore, R. (2013). Harmonisation of the soil map of Africa at the continental scale. *Geoderma, 211–212*, 138–153.

Fazzini, M., Bisci, C., & Billi, P. (2015). The climate of Ethiopia. In P. Billi (Ed.). *Landscapes and landforms of Ethiopia* (pp. 65–87). Dordrecht: Springer Science + Business Media.

Flanagan, L. B., & Farquhar, G. D. (2014). Variation in the carbon and oxygen isotope composition of plant biomass and its relationship to water-use efficiency at the leaf- and ecosystem-scales in a northern Great Plains grassland. *Plant, Cell and Environment, 37*, 425–438.

Gole, T. W. (2015). *Coffee: Ethiopia's gift to the world. The traditional production systems as living examples of crop domestication, and sustainable production and an assessment of different certification schemes.* Addis Ababa, Ethiopia: Environment and Coffee Forest

Forum (ECFF).
Gonzalvez, A., Armenta, S., & de la Guardia, M. (2009). Trace-element composition and stable-isotope ratio for discrimination of foods with Protected Designation of Origin. *Trends in Analytical Chemistry, 28*(11), 1295–1311.
Habte, G., Hwang, I. M., Kim, J. S., Hong, J. H., Hong, Y. S., Choi, J. Y., ... Kim, K. S. (2016). Elemental profiling and geographical differentiation of Ethiopian coffee samples through inductively coupled plasma-optical emission spectroscopy (ICP-OES), ICP-mass spectrometry (ICP-MS) and direct mercury analyzer (DMA). *Food Chemistry, 212*, 512–520.
Kelly, S., Heaton, K., & Hoogewerff, J. (2005). Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. *Trends in Food Science and Technology, 16*, 555–567.
Klingele, R. (1998). Hararghe farmers on the cross-roads between subsistence and cash economy. http://www.africa.upenn.edu/eue_web/hararghe.htm (Accessed 20.02. 2017).
Krivan, V., Barth, P., & Morales, A. F. (1993). Multielement analysis of green coffee and its possible use for the determination of origin. *Microchimica Acta, 110*, 217–236.
Liu, H.-C., You, C.-F., Chen, C.-Y., Liu, Y.-C., & Chung, M.-T. (2014). Geographic determination of coffee beans using multi-element analysis and isotope ratios of boron and strontium. *Food Chemistry, 142*, 439–445.
Luykx, D. M. A. M., & van Ruth, S. M. (2008). An overview of analytical methods for determining the geographical origin of food products. *Food Chemistry, 107*, 897–911.
McComb, J. Q., Rogers, C., Han, F. X., & Tchounwou, P. B. (2014). Rapid screening of heavy metals and trace elements in environmental samples using portable X-ray fluorescence spectrometer, a comparative study. *Water, Air, and Soil Pollution, 225*, 2169. https://doi.org/10.1007/s11270-014-2169-5.
McLeod, R. J., Garland, M., Hale, R. V., Steiman, S., & Frew, R. D. (2013). Determining the most effective combination of chemical parameters for differentiating the geographic origin of food products: An example using coffee beans. *Journal of Food Chemistry and Nutrition, 01*(02), 49–61.
Mehari, B., Redi-Abshiro, M., Chandravanshi, B. S., Combrinck, S., Atlabachew, M., & McCrindle, R. (2016a). Simultaneous determination of alkaloids in green coffee beans from Ethiopia: Chemometric evaluation of geographical origin. *Food Analysis Methods, 9*, 1627–1637.
Mehari, B., Redi-Abshiro, M., Chandravanshi, B. S., Combrinck, S., Atlabachew, M., & McCrindle, R. (2016b). Profiling of phenolic compounds using UPLC-MS for determining the geographical origin of green coffee beans from Ethiopia. *Journal of Food Composition and Analysis, 45*, 16–25.
Mehari, B., Redi-Abshiro, M., Chandravanshi, B. S., Combrinck, S., & McCrindle, R. (2016c). Characterization of the cultivation region of Ethiopian coffee by elemental analysis. *Analytical Letters, 49*, 2474–2489.
Moat, J., Williams, J., Baena, S., Wilkinson, T., Demissew, S., Challa, Z. K., ... Davis, A. P. (2017). *Coffee farming and climate change in Ethiopia: Impacts, forecasts, resilience and opportunities – Summary*. Kew (UK): The Strategic Climate Institutions Programme (SCIP), Royal Botanic Gardens37.
Muniz-Valencia, R., Jurado, J. M., Ceballos-Magana, S. G., Alcazar, A., & Diaz, J. H. (2014). Characterization of Mexican coffee according to mineral contents by means of multilayer perceptrons artificial neural networks. *Journal of Food Composition and Analysis, 34*, 7–11.
Rodrigues, C., Brunner, M., Steiman, S., Bowen, G. J., Noqueira, J. M., Gautz, L., ... Máguas, C. (2011). Isotopes as tracers of the Hawaiian coffee producing regions. *Journal of Agricultural and Food Chemistry, 59*, 10239–10246.
Rodrigues, C. I., Maia, R., Miranda, M., Ribeirinho, M., Nogueira, J. M. F., & Maguas, C. (2009). Stable isotope analysis for green coffee bean: A possible method for geographic origin discrimination. *Journal of Food Composition and Analysis, 22*, 463–471.
Rodrigues, W. P., Martins, M. Q., Fortunato, A. S., Rodrigues, A. P., Semedo, J. N., Simões-Costa, M. C., ... Ramalho, J. C. (2016). Long-term elevated air [CO2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *C. canephora* species. *Global Change Biology, 22*(1), 415–431.
Sereke-Brhan, H. (2010). Coffee, culture, and intellectual property: Lessons for Africa from the Ethiopian fine coffee initiative. The pardee papers/No. 11/July 2010. The Frederick S. Pardee Center for the Study of the Longer-Range Future, Boston University.
Serra, F., Guillou, C. G., Reniero, F., Ballarin, L., Cantagallo, M. I., Wieser, M., ... Vanhaecke, F. (2005). Determination of the geographical origin of green coffee by principal component analysis of carbon, nitrogen and boron stable isotope ratios. *Rapid Communications in Mass Spectrometry, 19*, 2111–2115.
Tefera, A., Tefera, T., & Gray, Q. (2014). *Coffee annual report.* Global Agricultural Information Network (GAIN) report number: ET 1402, 13 May 2014.
Tegegne, H. K. (2017). *Impact of deforestation on biodiversity, soil carbon stocks, soil quality, runoff, and sediment yield at southwest Ethiopia's forest frontier.* PhD ThesisGhent University, Belgium.
Teketay, D. (1999). History, botany and ecological requirements of coffee. *Walia, 20*, 28–50.
Teuber, R. (2007). *Geographical indications of origin as a tool of product differentiation – The case of coffee. No. 33*. Germany: Institute of Agricultural Policy and Market Research, University of Giessen.
Valentin, J. L., & Watling, R. J. (2013). Provenance establishment of coffee using solution ICP-MS and ICP-AES. *Food Chemistry, 141*, 98–104.
Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S* (4th ed.). New York: Springer.
Yeh, H.-W., & Wang, W.-M. (2001). Factors affecting the isotopic composition of organic matter: (1) Carbon isotopic composition of terrestrial plant materials. *Proceedings of the National Science Council, Republic of China, 25*(3), 137–147.
Yilma, T. (2001). Coffee-ensete-livestock interaction for sustainable livelihood in the Sidama area of southern Ethiopia. *Journal of Agriculture in the Tropics and Subtropics, 102*(2), 119–131.